DATA EVALUATION RECORD
SEMI-FIELD POLLINATOR STUDY
(NON-GUIDELINE STUDY)

1. **CHEMICAL:** Boscalid — PC Code No.: 128008

2. **TEST MATERIAL:** Pristine®
Boscalid (BAS 510F) Purity: 25.2%
CAS 188425-85-6
Pyraclostrobin (BAS 500F) Purity: 12.8%
CAS

3. **CITATION**

Authors: Jessica Lawrence and MaryEllen Riley
Title: Determination of Residues of Pristine Fungicide (Pyraclostrobin + Boscalid) in Royal Jelly and Pollen in Almond Trees in Central California.

Study Completion Date: November 30, 2012

Laboratory: Eurofins Agroscience Services, Inc., Cedar Grove Research Facility, 8909 Atkins Rd., Mebane, NC 27302

Sponsor: BASF Corporation, 26 Davis Drive, P.O. Box 13528, Research Triangle Park, NC 27709

Laboratory Report ID: Eurofins Study Code: S12-00011; BASF Study ID: 373827

MRID No.: 490093-04
DP Barcode: D408124

4. **REVIEWED BY:** Thomas Steeger, Ph.D., Senior Biologist, ERB 4, Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Signature: Thomas Steeger 3/30/13 **Date:** 03/30/13

5. **REVIEWED BY:** Catherine Aubee, Biologist, ERB 4, Environmental Fate and Effects Division, U. S. Environmental Protection Agency

Signature: C Aubee **Date:** 30 March 2013

6. STUDY PARAMETERS

Test Species: Honeybee: *Apis mellifera*
Age of Test Organisms at Test Initiation: Adult and bee brood (larvae or eggs).
Test Duration: 12 Days

7. CONCLUSIONS:

The formulated product Pristine® consisting of boscalid (BAS 510 F; 25.2%) and pyraclostrobin (BAS 500F; 12.8%), was applied to almond trees at a rate of 13.88 oz of formulated product per acre (equivalent to a rate of 0.22 lbs ai/A boscalid and 0.11 lbs ai/A pyraclostrobin) using an airblast sprayer. The application included the non-ionic low foam wetter/spreader adjuvant Induce®. Immediately following application, almond trees (approximately 4 years of age and at roughly 30% bloom) were enclosed in a fine mesh tunnel. Samples (~1 g) of wax, bee bread, nectar, honey and pollen were collected the day prior to application (-1) as well as 1, 3, 4, 6, 7, 9 and 12 days after application (DAA) and were analyzed for boscalid and pyraclostrobin residues using liquid chromatography coupled with tandem mass spectroscopy.

The available data indicate that measurable quantities of both boscalid and pyraclostrobin were detected in almond blossoms, pollen, and bee bread up to 12 DAA; however, residues in honey, royal jelly, queen larvae and nectar were below the LOQ (<0.02 ppm) throughout the study period. Residues of both active ingredients were at or near the LOQ in wax throughout the study period. No residues are reported on the day of application (0 DAA)for the various matrices sampled. Residues of both boscalid and pyraclostrobin in almond blossoms declined by roughly an order of magnitude from 1 DAA (28.8 and 14.7 ppm, respectively) to 7 DAA (3.43 and 1.27 ppm, respectively). Residues of boscalid in pollen declined by a factor of 3x from 3DAA (3.08 ppm) to 12 DAA (1.37 ppm) while residues of pyraclostrobin declined by roughly a factor of 5x (1.53 ppm to 0.29 ppm) over the same sampling period. Although residues in honey were below the LOQ over the sampling period, residues in bee bread were increasing from 3 to 12 DAA; mean boscalid residues in bee bread increased from 0.08 to 0.41 ppm while mean pyraclostrobin residues increased from 0.04 to 0.14 ppm from 3 to 12 DAA.

This is a non-guideline study and is classified as supplemental. The study suggests that treatment of almond trees at 30% bloom with Pristine® results in detectable residues of both boscalid and pyraclostrobin in almond blossom and almond pollen up to 12 DAA; however, these residues were declining over the 12-day study period. The data also indicate that the residues were primarily associated with pollen and not nectar. Residues in bee bread actually increased over the study period and there is uncertainty as to the extent that this increase in residue level would continue. Residue levels in royal jelly and in queen larvae were below the LOQ; however, there is uncertainty whether increasing residues in bee bread may ultimately have translated to royal jelly since nurse bees are likely consuming bee bread and not pollen directly. No data are provided for pollen during the first two days of the study and there is uncertainty as to the extent of exposure that may have occurred when residues were at there highest in almond blossoms.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: This is a non-guideline study examining residues of boscalid and pyraclostrobin

in various hive matrices. The formulated product (Pristine®) was not applied while bees were actively foraging and there is uncertainty as to the extent that residues may have differed had bees been actively foraging. Untreated colonies (UTC) were not treated with adjuvant Induce®; ideally, the controls should be treated in the same manner as the treatment groups minus the use of Pristine®.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

Target application rate was 14.5 oz Pristine®/A (0.23 lbs boscalid/A plus 0.12 lbs pyraclostrobin/A); actual application rate was 13.88 oz Pristine®/A (0.22 lbs boscalid/A plus 0.11 lbs pyraclostrobin/A). Although 1 – 5 g sample sizes were targeted for the various matrices sampled, the researchers were not always able to collect the sample presumably due to insufficient quantities of the material in the colony.

10. SUBMISSION PURPOSE:

According to the study authors, the objective of this study was to determine the range of residues of boscalid and pyraclostrobin in almond blossoms and several honey bee matrices following application of Pristine® fungicide to almond trees in bloom in Central California. The study was submitted in response to previous ecological risk assessment recommendations (DP Barcode 363523 *et seq*.) to examine effects on honey bee queen cell development.

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11. MATERIALS AND METHODS

A. Test Organisms: The honeybee, *Apis mellifera* nuclei [colonies]; obtained from beekeeper in Rheinland-Pfalz, Germany).

B. Test Design:

The study consisted of 4 treated tunnels and 4 untreated tunnels. Each replicate consisted of 11 trees (~14 in height) in a row extending 198 ft. The test crop consisted of almond trees (Non-Pareil variety) at 30% bloom or BBCH 63 (**B**undesanstalt, **B**undessortenamt und **CH**emische Industrie; BBCH scale used to represent the phenological development stages of plants) growth stage. Replicates were positioned in series with all of the untreated control (UTC) colonies followed by the treated controls. UTC replicates 1 and 2 abutted one another and were separated from UTC replicates 3 and 4 by a 18-ft buffer. Treatment replicates were separated from UTC replicate 4 by a 108-ft buffer; treatment replicates 1 and 2 abutted one another and were separated from treatment replicates 3 and 4 by a 19-ft buffer. The tented enclosures were arranged north-south since the prevailing wind was out of the northwest.

Each tunnel contained two honey bee colonies; one standard 10-frame nucleus [queen-right] colony and a second smaller 5-frame colony that was queenless and used to collect royal jelly and larval queens. The larger queen-right colony was used to collect wax, bee bread, nectar, honey and pollen. Samples were collected the day prior to application (-1) as well as 1, 3, 4, 6, 7, 9 and 12 days after application (DAA). At each sampling event, hive matrices sampled (~ 1 gram) included: almond blossoms, larval queens, pollen basket contents, bee bread, royal jelly, was, nectar and/or honey; however, pollen samples were below the 1 g target. Grafting of larvae from the "mother hive" to the grafting frames of the nucleus

colony was performed approximately 3 days before royal jelly was sampled from the smaller colony

Field spikes were included at DAA1 and DDA9; nectar and pollen were spiked to a nominal concentration of 50 µg/g (ppm) for boscalid and pyraclostrobin each. Pre-weighed samples (blossoms, larval queens, pollen basket contents, bee bread, royal jelly, wax, nectar and/or honey) were placed in plastic containers and frozen promptly after collection. Residues of boscalid and pyraclostrobin were quantified using liquid chromatography, tandem mass spectroscopy (LC/MS/MS). The limit of quantification (LOQ) for both compounds was 0.02 mg/kg (parts per million; ppm); the limit of detections (LOD) was 20% of the LOQ or 0.004 ppm for boscalid and pyraclostrobin.

According to the study methods, all of the colonies used in the study were from a Eurofins' apiary, and all colonies were treated with the varroacide Apiguard® (thymol) just before study initiation. Colonies were acclimated to the enclosures with high density polyethylene tread covering four days prior to application of the test chemical. The evening before application the hives were sealed and covered in plastic and the mesh enclosures on the 4 treated replicates were pulled back to allow treatment the following day. The next morning a single foliar (spray blast) application of the test material was made to the trees at a rate of 13.88 oz product (0.22 lbs boscalid/A and 0.11 lbs pyraclostrobin/A). The application included the non-ionic low foam wetter/spreader adjuvant Induce® (reported as a proprietary blend of [redacted]) since it was reported as grower standard practice to use an adjuvant in combination with Pristine . Mesh covering control replicates was not pulled back.

12. <u>REPORTED RESULTS:</u>

According to the report, beebread samples were not available from all of the UTCs at 7 DAA and 12 DAA and were only available from one treated replicate at 12 DAA. At 3DAA and 12DAA, honey was not available for sampling from all of the UTC; honey was not available for sampling from all of the treated replicates at 7 DAA or at 12 DAA. Nectar could not be sampled from all of the UTC at 7DAA or 12 DAA. Larval queens were not available from all treated plots at -1DAA, 6DDA and 9DDA. Pollen was not available from all untreated and treated plots at all sampling events, and at 1DAA and 2DAA no pollen samples were collected from treated plots. Royal jelly was not available from all treated plots at -1DAA, 6DAA, and 9DAA.

Residue levels of boscalid and pyraclostrobin were below the LOQ (<0.02 ppm, each) in/on UTC samples except for one wax sample with pyraclostrobin at 0.05 ppm 12 DAA and one pollen sample with boscalid at 0.02 ppm 12 DAA. **Table 1** summarizes the mean percent recoveries (±standard deviation) for boscalid and pyraclostrobin in the various matrices sampled. Field spikes were stored for 119 to 131 days prior to analysis.

Table 16. Mean percent recoveries (± standard deviation) for boscalid and pyraclostrobing in matrices sampled during the Pristine residue study in almond trees.

Matrix	Boscalid (mean ± std dev) %	Pyraclostrobin (mean ± std dev) %
Almond blossoms	103 ±12	93 ± 6
Pollen	106 ± 31	95 ± 23
Bee Bread	93 ± 11	118 ± 10
Nectar	92 ± 12	101 ± 23
Wax	80 ± 12	89 ± 20
Honey	97 ± 7	102 ± 9

Table 2 summarizes mean residues of boscalid and pryraclostrobin in matrices sampled at 1, 2, 3, 7 and 12 DAA.

Table 17. Mean residues and associated ranges (in ppm) of boscalid and pyraclostrobin in matrices sampled at 1, 2, 3, 7 and 12 days after application (DAA) of Pristine to almond trees.

Matrix	Sample Period (DAA)	Boscalid Mean (range) ppm	Pyraclostrobin Mean (range) ppm
Almond Blossom	1	28.8 (22.3 – 36.4)	14.7 (11.7 – 18.4)
	2	19.3 (18.9 – 19.7)	9.17 (8.72 9.66)
	3	17.7 (13.3 – 25.4)	8.38 (6.40 – 11.8)
	7	3.43 (3.84 – 3.97)	1.27 (1.05 - 1.49)
	12	--	--
Almond Pollen	1	--	--
	2	--	--
	3	3.08 (2.37 – 3.98)	1.53 (1.15 – 1.88)
	7	2.16 (0.51 – 4.16)*	0.71 (0.17 – 1.32)*
	12	1.37 (1.01 – 1.69)	0.29 (0.21 – 0.36)
Bee Bread	1	--	--
	2	--	--
	3	0.08 (0.03 – 0.13)	0.04 (<0.02 – 0.07)
	7	0.26 (0.04 – 0.39)	0.09 (<0.02 – 0.16)
	12	0.41	0.14
Honey	1	--	--
	2	--	--
	3	<0.02	<0.02
	7	<0.02	<0.02
	12	<0.02	<0.02
Royal Jelly	1	--	--
	2	--	--
	3	<0.02	<0.02
	7	<0.02**	<0.02**
	12	<0.02***	<0.02***
Queen Larvae	1	--	--
	2	--	--
	3	<0.02	<0.02
	7	<0.02**	<0.02**
	12	<0.02***	<0.02***
Nectar	1	<0.02	<0.02
	2	<0.02	<0.02

	3	<0.02	<0.02
	7	<0.02	<0.02
	12	<0.02	<0.02
Wax	1	<0.02	<0.02
	2	0.04	0.02
	3	0.04	0.05
	7	0.10	0.04
	12	0.04	<0.02

¯ No sample collected
* sampled 8DAA
** sampled 6DAA
*** sampled 9DAA

13. REVIEWER COMMENTS:

The authors report that Apiguard® (thymol) was used just prior to the initiation of the study. According to the leaflet[25] published by Dadant, while the product can be used in the springtime, it can sometimes make the queen stop egg laying.

The methods section does not describe how the UTC replicates were treated other than to note that the tunnel mesh was not pulled back and that the control colonies were covered/sealed for the same period as treated colonies. However, minimally, the UTC should have been sprayed with Induce.

Although the methods section indicates specific sampling days when various matrices could not be sampled, the presumption is that while samples could not be collected from "all" of the treated and/or UTC replicates, at least some replicates could be sampled. The only exception was presumably at 1 and 2DAA when "no" pollen was available for sampling from treated plots.

Although the executive summary states that the minimum targeted sample sizes of 1 gram, the methods section states that "[t]argeted sample sizes varied for each matrix, consisting of a minimum of 5 g of blossoms, 1 gram of pollen, 1 gram per hive of bee bread, 1 g per hive of nectar, 5 g of honey, 1 g per hive of wax, and 1-3 g of royal jelly per replicate plot at each sampling event. However, minimum sample size was not always met for pollen and royal jelly."

The targeted sample sizes of `1 – 5 gram and the acknowledged difficulty in collecting sufficient samples across the various sampling matrices suggests that the capacity of the study replicates to support bee foraging activity may have been limited by the size of the replicates in terms of the number and size of trees as well as the extent of bloom.

A certificate of analysis is provided for the test material applied; however, no data are provided to substantiate the actual amount applied to almond trees. Based on the data presented in **Table 2** for residues of boscalid and pyraclostrobin, no data are available for almond blossom samples 12 DAA; no data are available for pollen, bee bread, honey, royal jelly or queen larvae at 1 and 2 DAA. The available data indicate that measurable quantities of both boscalid and pyraclostrobin were detected in almond blossoms, pollen, and bee bread up to 12 DAA; however, residues in honey, royal jelly, queen larvae and nectar were below the LOQ (<0.02 ppm) throughout the study period. Residues of both active ingredients

[25] http://www.dadant.com/wp-content/uploads/2011/09/Apiguard-QA.pdf

were at or near the LOQ in wax throughout the study period. Residues of both boscalid and pyraclostrobin in almond blossoms declined by roughly an order of magnitude from 1 DAA (28.8 and 14.7 ppm, respectively) to 7 DAA (3.43 and 1.27 ppm, respectively). Residues of boscalid in pollen declined by a factor of 3x from 3DAA (3.08 ppm) to 12 DAA (1.37 ppm) while residues of pyraclostrobin declined by roughly a factor of 5x (1.53 ppm to 0.29 ppm) over the same sampling period. Although residues in honey were below the LOQ over the sampling period, residues in bee bread were increasing from 3 to 12 DAA; mean boscalid residues in bee bread increased from 0.08 to 0.41 ppm while mean pyraclostrobin residues increased from 0.04 to 0.14 ppm from 3 to 12 DAA.

It is unclear why no residue data are reported for almond blossoms12 DAA; however, residue levels are reported for pollen so presumably the trees were still flowering at this time. It is unclear why data are not available for pollen at 1 and 2 DAA and this absence of pollen was reflected in the absence of bee bread over these two sampling days. It is uncertain though if there was no honey, pollen and/or bee bread available in the colony during the first two days of the study, what hive bees may have been reliant on for food during this period.

In general, residue levels in royal jelly and in queen larvae were below the LOQ; however, there is uncertainty whether increasing residues in bee bread may ultimately have translated to royal jelly since nurse bees are likely consuming bee bread and not pollen directly. No data are provided for pollen during the first two days of the study and there is uncertainty as to the extent of exposure that may have occurred when residues were at there highest in almond blossoms.